(For Laboratory Use Only)
ATS Labs Project # A16746
SCC 5-27-14

ATS LABS

PROTOCOL

Fungicidal Use-Dilution Method

Test Organism:

Trichophyton mentagrophytes (ATCC 9533)

PROTOCOL NUMBER

KIK02033114.FUD

PREPARED FOR

KIK International, Inc
909 Magnolia Avenue
Auburndale, FL 33823

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

DATE

March 31, 2014

EXACT COPY
INITIALS JW DATE 6/25/14

Fungicidal Use-Dilution Method

SPONSOR: KIK International, Inc
909 Magnolia Avenue
Auburndale, FL 33823

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The purpose of this study is to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the following: The U.S. Environmental Protection Agency (EPA).

TEST SUBSTANCE CHARACTERIZATION
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized by the Sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is April 17, 2014. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of May 15, 2014. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs nor any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the regulatory agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
Regulatory Agencies require that a specific organism claim for a test substance intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed organism. This is accomplished in the laboratory by treating the target organism with the test substance under conditions which simulate as closely as possible the actual conditions under which the test substance is designed to be used. For products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting data. The experimental design in this protocol meets these requirements. Appropriate modifications including test system propagation and carrier drying may have been made for test organisms not defined in the published test methodology.

TEST PRINCIPLE

A film of organism cells dried on a surface of stainless steel carriers is exposed to the test substance for a specified exposure time. After exposure, the carriers are transferred to vessels containing neutralizing subculture media and assayed for survivors. Appropriate culture purity, sterility, viability, carrier population and neutralization confirmation controls are performed. The current version of Standard Operating Procedure CGT-4090 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Trichophyton mentagrophytes	9533	Sabouraud Dextrose Agar or Glucose Agar	25-30°C, aerobic

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Carriers

Carriers will be screened according to AOAC Official Method of Analysis and any carrier positive for growth will be discarded. Only penicylinders showing no growth may be used. Stainless steel penicylinders will be pre-soaked overnight in 1N NaOH, washed in water until neutral and autoclaved in deionized water. Carriers shall be used within three months of sterilization.

Preparation of Test Organism

A culture of *Trichophyton mentagrophytes* will be prepared by inoculating a sufficient number of agar plates using a stock culture and incubating at 25-30°C for 10-15 days. The mycelia will be removed from sufficient plates using a sterile device. The mycelia will be transferred to sterile glassware (e.g. an Erlenmeyer flask) containing glass beads and a ratio of 25 mL of sterile saline or saline/Triton Solution (0.85% Saline + 0.05 % Triton X-100) per 5 plates harvested. The culture will be agitated. Alternately, the mycelia may be added to a tissue grinder containing sterile saline or saline/Triton Solution and macerated. The culture will be filtered through sterile gauze to remove hyphal fragments. The conidial concentration will be estimated by counting in a hemacytometer and the culture may be adjusted as necessary. The inoculum must contain at least 5 x 10^6 condia/mL. The culture may be stored at 2-8°C for up to 4 weeks prior to use in testing.

An organic soil load may be added to the test culture per Sponsor's request. The test culture will be thoroughly mixed prior to use.

Contamination of Carriers

The culture will be transferred to the penicylinders (after siphoning off the water) and the carriers will be immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture to completely cover the carriers. A maximum of 100 carriers will be inoculated per vessel and each vessel inoculated may be considered a part of one total inoculation run per organism. The inoculated carriers will be transferred to sterile Petri dishes matted with filter paper after tapping the carrier against the side of the container to remove excess inoculum. No more than twelve carriers will be placed in each Petri dish. The carriers will be dried for 40±2 minutes at 35-37°C. *NOTE: Organisms not specifically mentioned in the AOAC methodology may require modified drying conditions for the purpose of obtaining maximum survival following drying. The actual drying conditions will be clearly documented.* Carriers will be used in the test procedure within 2 hours of drying. Carriers that touch during drying or have fallen over will not be used in the test.

Preparation of Test Substance

The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation. Ten (10) mL of the test substance at its use-dilution will be aliquotted into the required number of sterile 25 x 150 mm or 25 x 100 mm tubes. The tubes will be placed into a waterbath at the specified exposure temperature, and allowed to equilibrate for ≥10 minutes prior to testing.

Exposure Conditions
Each contaminated and dried carrier will be placed into a separate tube containing 10 mL of the test substance at its use-dilution for the desired exposure time and temperature. Immediately after placing each test carrier in the test tube, swirl the tube using approximately 2–3 gentle rotations to release any air bubbles trapped in or on the carrier. Care will be taken to avoid touching the sides of the tubes which may compromise exposure. The carrier will be placed into the test substance within ±5 seconds of the exposure time for exposure times above 1 minute following a calibrated timer. The carrier will be placed into the test substance within ±3 seconds of the exposure time for exposure times of ≤1 minute. If the exposure conditions are compromised in any way for a given carrier, a new carrier may be treated in its place. If this cannot be done, the carrier will be marked and the compromised carrier will be identified in the raw data. If a marked carrier demonstrates a positive result, the carrier set may be invalidated and repeated by Sponsor request.

Test System Recovery
Following the Sponsor specified exposure time, each medicated carrier will be transferred by wire hook at staggered intervals to 10 mL of primary neutralizing subculture medium and each tube will be shaken thoroughly. To accomplish this, the carrier is removed from the disinfectant tube with a sterile hook, tapped against the interior sides of the tube to remove the excess disinfectant, avoiding the upper one-third of the tube, and transferred into the subculture tube. Care will be taken to avoid excessive contact to the interior sides of the subculture tubes during transfer. If secondary neutralization is requested by the Sponsor or deemed necessary due to test substance active and/or concentration, carriers will be transferred into individual secondary subculture tubes containing 10 mL of neutralizing broth beginning approximately 25-60 minutes after subculture of the carrier into the primary neutralizing subculture medium. Shake each tube thoroughly. If neutralization is a concern, 20 mL of subculture medium may be used.

Incubation and Observation
All neutralized subcultures are incubated for 10 days at 25-30°C. The agar plate subcultures will be incubated for 44-76 hours at 25-30°C . Additional incubation may be followed for the subculture plates if growth is hard to detect.

Following incubation, the subcultures will be visually examined for growth. If necessary, the subcultures may be placed at 2-8°C for up to three days prior to examination.

Representative subculture tubes showing growth will be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
The serum used for soil load will be cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier will be added to the neutralizing subculture medium. The subculture medium containing the carrier will be incubated and examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Viability Control
One representative inoculated carrier will be added to a vessel containing each type of subculture medium. If secondary subcultures are performed using a different media type, one carrier will be placed in the primary subculture medium and one carrier will be placed in the secondary subculture medium. The vessels containing each carrier will be incubated and visually examined for growth. The acceptance criterion for this study control is growth in the subculture media.

Neutralization Confirmation Control
Prior to testing or concurrent with testing, the neutralization of the test substance will be confirmed by exposing at least one sterile carrier to the test substance and transferring the carrier to primary subcultures containing 10-20 mL of neutralizing subculture medium as in the test. If performed in the test procedure, each carrier will then be transferred from primary subcultures into individual secondary subcultures beginning approximately 25-60 minutes following the primary transfer. The subcultures (primary and secondary as applicable) will be inoculated with a target of 10-100 colony forming units (CFU) of each test organism, incubated under test conditions and visually examined for the presence of growth. This control will be performed with multiple replicates using different dilutions of the test organism. A standardized spread plate procedure will be run concurrently in order to enumerate the number of CFU actually added per tube. NOTE: Only the most concentrated test substance and/or shortest exposure time needs to be evaluated in this control.

The acceptance criterion for this study control is growth in the final subculture broth, minimally, following inoculation with ≤100 CFU per tube.

Carrier Population Control
Two sets of three inoculated carriers (one set prior to testing and one set following testing) for each organism carrier set will be assayed. Each inoculated carrier will be individually subcultured into a tube containing 10 mL of neutralizing subculture medium and sonicated for 1 minute±5 seconds. Tubes will be contained in a beaker with water suspended in the ultrasonic cleaner such that all fluids will be level. Following sonication, the contents of the three subcultured carriers will be pooled (30 mL) and briefly vortex mixed. Appropriate serial ten-fold dilutions will be prepared and the duplicate aliquots spread plated on agar plate medium, and incubated. If serial dilutions are not performed and plated immediately following sonication, the vessels may be refrigerated at 2-8°C for up to two hours prior to dilution. Following incubation, the resulting colonies will be enumerated and the CFU per carrier set calculated. The individual CFU per carrier set results will be calculated, and the Log_{10} value of each carrier set determined. The average Log_{10} value per organism will be calculated. The acceptance criterion for this study control is a minimum average Log_{10} value of 4.0.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The efficacy performance requirements for label claims state that the test substance must kill the microorganism on 10 out of the 10 inoculated carriers.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number. If the population control exceeds an average log_{10} value of 5.0 for *Trichophyton mentagrophytes*, and the test substance does not meet the performance criteria, the Sponsor may invalidate the study and repeat testing.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the fungal strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the current effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official Method 964.02, Testing Disinfectants against *Pseudomonas aeruginosa* - Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
2. Association of Official Analytical Chemists (AOAC) Official Method 955.15, Testing Disinfectants against *Staphylococcus aureus* - Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
3. Association of Official Analytical Chemists (AOAC) Official Method 955.14, Testing Disinfectants against *Salmonella enterica*- Use-Dilution Method. In Official Methods of Analysis of the AOAC, 2013 Edition.
4. Association of Official Analytical Chemists (AOAC) Official Method 960.09, Germicidal and Detergent Sanitizing Action of Disinfectants Method [Preparation of Synthetic Hard Water]. In Official Methods of Analysis of the AOAC, 2013 Edition.
5. Association of Official Analytical Chemists (AOAC) Official Method 961.02, Germicidal Spray Products as Disinfectants. In Official Methods of Analysis of the AOAC, 2012 Edition.
6. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.
7. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.

DATA ANALYSIS

Calculations

Determine the CFU/Carrier set in the Carrier Population Control using all average counts between 0-300 CFU as follows:

$$\text{CFU/carrier} = \frac{[(\text{avg. CFU for } 10^{-x}) + (\text{avg. CFU for } 10^{-y}) + (\text{avg. CFU for } 10^{-z})] \times (\text{Volume of neutralizer})}{[10^{-x} + 10^{-y} + 10^{-z}] \times (\text{Volume plated}) \times (\#\text{ of carriers per set})}$$

where 10^{-x}, 10^{-y}, and 10^{-z} are example dilutions that may be used

$$\text{Average Log}_{10}\text{ Carrier Population Control} = \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \ldots\text{Log}_{10}X_N}{N}$$

Where: X equals CFU/carrier set
N equals number of control carrier sets

Statistical Analysis

None used

STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number) exactly as it should appear on final report:
TECOMSEH B EPA REG. NO.: 70271-24
LOT #'S 14091231SM3FL01 AND 14093140GM3FL01

The requirement to test at the lower certified limit (LCL) for registration has not yet been defined for this organism by the U.S. EPA; however, testing at the LCL is recommended due to agency uncertainty.

Product Description:

- ☐ Quaternary ammonia
- ☐ *Iodophor*
- ☑ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ *Peroxide*
- ☐ Other_____

Test Substance Active Concentration (upon submission to ATS Labs): ~8.25%

Neutralization/Subculture Broth: ☐ _____

(NOTE: All broth must also serve as an appropriate growth medium for the test organism)

☑ ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions:

- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other:_____

Hazards:

- ☐ None known: Use Standard Precautions
- ☑ Material Safety Data Sheet, Attached for each product
- ☐ As Follows:_____

Product Preparation

- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilution(s) to be tested:
  - 1/2 cup per gallon (example: 1 oz/gallon) defined as 1/2 cup (amount of test substance) + 1 gallon (amount of diluent)
  - ☐ Deionized Water (Filter or Autoclave Sterilized)
  - ☑ Tap Water (Filter or Autoclave Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____PPM
  - ☑ Other See modification

Note:** **An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism: *Trichophyton mentagrophytes* (ATCC 9533)

Carrier Number: 10 per batch

Exposure Time: 5 Minutes **Exposure Temperature:** 20 ± 1 °C

Organic Soil Load:

- ☐ Minimum 5% Organic Soil Load (fetal bovine serum)
- ☑ No Organic Soil Load Required
- ☐ Other_____

TEST SUBSTANCE SHIPMENT STATUS

- ☐ Has been used in one or more previous studies at ATS Labs .
- ☐ Has been shipped to ATS Labs (but has not been used in a previous study).
  Date shipped to ATS Labs: _____ Sent via *overnight* delivery? ☐ Yes ☐ No
- ☑ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs: 4/17/2014
- ☐ *Sender (if other than Sponsor):* _____

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

- ☐ Approved without modification
- ☑ Approved with modification

Prior to each testing date, titrate the test substance per ATS Labs SOP CGT-0090 to determine starting sodium hypochlorite concentrations. Dilute test substance to the Lower Certified Limit of 67,000-67-500 ppm with sterile tap water and titrate to confirm. Dilute test substance per page 8 of protocol for use in testing.

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Mr. Justin Lowe TITLE: Regional QA Manager

SIGNATURE: Justin Lowe DATE: 4/4/2014

PHONE: (863) 551 - 3006 FAX: _____ EMAIL: jlowe@kikcorp.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

ATS Labs:

NAME: Jill Ruhmel
Study Director

SIGNATURE: Jill Ruhmel DATE: 5-2-14
Study Director